REMARKS

Claims 13-25 are pending in the application and are rejected. Claims 13, 16, 21 and 24 are herein amended. Claims 14, 15, 17-20, 22, 23 and 25 are herein cancelled without prejudice. No new matter has been added. Applicants thank Examiner Ricci for the courtesies extended in the telephone interview of February 16, 2012. Applicants' Statement of the Substance of the Interview is incorporated herein.

Information Disclosure Statement

Applicants note that the most recent Office Action does not include an SB/08 form in which Tanaka et al. is initialed as having been considered, as previously requested. Applicants respectfully request that this be provided in the next Office communication.

Applicants' Response to Claim Rejections under 35 U.S.C. §112

Claims 13-16 and 21-25 are rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement.

It appears to be the position of the Office Action that the specification does not provide support for the full scope of the claims. Applicants note that on pages 3 and 4 of the Office Action, extensive comments are provided mainly directed at a genus/species situation.

In particular, the Office Action cites *Ariad* on page 3, stating "a generic claim may define the boundaries of a vast genus of chemical compounds, and yet the question may still remain

whether the specification, including original claim language, demonstrates that the applicant has invented species to support a claim to a genus."

As discussed in the telephone interview, it is improper to consider the "test compounds" as a genus. In particular, Applicants refer to Claim 2 of Example 17 of the USPTO Written Description Guidelines (http://www.uspto.gov/web/menu/written.pdf), which is a similar type of claim. An excerpt of the Guidelines is attached.

Applicants respectfully submit that the specification fully supports the claimed methods which recite the measurement of nuclear translocation of a single protein (Rac) in a single cell type (HUVEC). Applicants note that the claims do not recite measurement of, for example, nuclear translocation of a broad class of proteins in a broad class of cell types.

Applicant note that many comments on pages 6 and 7 of the Office Action are actually issues relating to enablement requirement, and have no bearing on the question of compliance with the written description requirement. Applicants respectfully submit that, based the disclosure, the inventors very clearly conveyed their invention to those skilled in the art.

In view of the above, Applicants respectfully request that the written description rejection be withdrawn. Additionally, however, Applicants here amend the claims to recite the "vascular cell disorder" in greater detail (claim 17) and to recite the "function of Rac" in greater detail (claim 21). Favorable reconsideration is respectfully requested.

Claims 13-16 and 21-25 are rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the enablement requirement.

It is the position of the Office Action that the claims are not enabled. The Office Action relies on many of the same comments as in the written description rejection. In particular, Applicants note the comment in paragraph 17 on pages 10-11 of the Office Action. The Office Action states that "Applicant's data do not demonstrate that the observed translocation of Rac into the nucleus is in any way responsible for pitavastatin's therapeutic effects." However, this is the very point that Masamura addressed.

On this point, in the telephone interview, the Examiner stated that the experimental data may demonstrate a "downstream event" rather than a so-called a "critical event." It was agreed that Rac1 which is localized to the nucleus cannot have its negative effects. However, the Examiner stated that it is unclear if there is enough translocation of Rac1 such a NADPH oxidase has reduced activity.

The Examiner suggested amending the claims to recite, for example, measuring transfer of the fusion protein from the cell membrane or cytosol into the nucleus. As such, Applicants herein amend the claims to recite "measuring distribution of the amount of fluorescence" and to recite, for example, "determining that the test substance is a substance which inhibits the function of Rac protein if the distribution of fluorescence in the nucleus of said first HUVEC culture is greater than the distribution of fluorescence in the nucleus of said second HUVEC culture by a significant amount." Applicants respectfully submit that this includes measuring the transfer of the fusion protein from the cell membrane or cytosol into the nucleus.

The pending claims recite two HUVEC cultures: a first HUVEC culture which is subjected to a test substance, and a second HUVEC culture which is a control. A comparative

point for observation is a distribution state of the fluorescence in the cell. Measuring the distribution of fluorescence includes measuring the amount of fluorescence in the membrane and the entire cytoplasm as compared with the amount of fluorescence in the nucleus. Since the amount of total fluorescence will be constant, the distribution of the fluorescence may be observed by microscope and can be illustrative of translocation and activity of Rac. In other words, Applicants respectfully submit that in order for the fluorescence of the fusion protein to be observed in the nucleus, it must be removed from the cytoplasm and cell membrane. One having ordinary skill in the art can easily make and use the claimed screening methods. Therefore, Applicants respectfully submit that enablement rejection should be withdrawn. Favorable reconsideration is respectfully requested.

Applicants' Response to Claim Rejections under 35 U.S.C. §103

Claims 17-20 are rejected under 35 U.S.C. §103(a) as being unpatentable over Krall et al. (Infection and Immunity 70: 360-367, 2002) in view of Essler et al. (Cellular Signalling 14:607-613, 2002).

It is the position of the Office Action that Krall discloses the embodiments as claimed, with the exception of teaching that the methods take place in HUVEC cells. The Office Action relies on Essler to provide this teaching.

Krall is directed to *In vivo* Rho GTPase-activating activity of *Pseudomonas aeruginosa* cytotoxin ExoS. As identified by Krall, Rac is a Rho GTPase that is involved in maintaining the actin cytoskeleton of cells. *Pseudomonas aeruginosa* is a bacterium which produces several

cytotoxins, including ExoS. Krall studies the effect of ExoS on Rho GTPases including Rac. See introduction on page 360. As discussed in the Materials and Methods section on page 361, Krall discusses creation of a GFP-Rac1 fusion protein ("Plasmid Construction"). Additionally, Krall discusses determining the cellular location of Rho GTPases by fluorescence microscopy ("Cellular location of Rho GTPases and measurement of the effects of dominant active Rho GTPases"). As discussed on page 362, when the GFP-Rac1 fusion protein was transfected into CHO cells (Chinese hamster ovary cells), "GFP-Rac1 localized to the plasma membrane, nuclear region, and cytosol with transfected cells showing membrane ruffling." First column, first full paragraph, lines 8-10. These cells also had enhanced actin stress fibers, indicating that Rac1 was in its activated state. In order to determine the effect of ExoS on the transfected cells, ExoS was co-expressed with the GFP-Rac1. Krall found that when ExoS was not expressed, GFP-Rac1 was mostly localized in membranes, but when ExoS was expressed, GFP-Rac1 re-localized from the membranes to the cytosol.

Essler discloses that sphingosine 1-phosphate (S1P) dynamically regulates myosin light chain phosphate activity in human endothelial cells. In particular, Essler discloses an activation assay of Rac in HUVEC cells. As discussed on page 611, when not stimulated by S1P, Rac is located in the cytosol of the HUVEC cells. However, when stimulated by S1P, Rac briefly translocates to peripheral membranes, and then returns to the cytosol.

First, Applicants respectfully submit that there is no reason provided as to why it would have been obvious to substitute HUVEC cells for CHO cells. Applicants respectfully submit that the rejection is improper for at least this reason.

Next, Applicants respectfully submit that neither Krall nor Essler discloses or suggests "measuring distribution of the amount of fluorescence in said first HUVEC culture and said second HUVEC culture" and, for example, "determining that the test substance is a substance which inhibits the function of Rac protein if the distribution of fluorescence in the nucleus of said first HUVEC culture is greater than the distribution of fluorescence in the nucleus of said second HUVEC culture by a significant amount."

In Krall, it is disclosed that the GFP-Rac1 is initially located in the plasma membrane, nuclear region and cytosol. Krall discloses observation of translocation of Rac1 between the cytosol and plasma membrane. Similarly, Essler discloses observation of Rac translocating between the cytosol and peripheral membranes. Thus, Applicants respectfully submit that neither of these references discloses measuring distribution of the amount of fluorescence in said first HUVEC culture and said second HUVEC culture and, for example, determining that the test substance is a substance which inhibits the function of Rac protein if the distribution of fluorescence in the nucleus of said first HUVEC culture is greater than the distribution of fluorescence in the nucleus of said second HUVEC culture by a significant amount.

Additionally, Applicants respectfully submit that the cited art is different from the claimed embodiments, since the cited art demonstrates observations of activated Rac1 behavior after been already activated. Neither of the cited art references actually discloses measuring distribution of fluorescence of a Rac1 fusion protein in response to culturing with a test substance. In particular, Applicant note that the claims recite using a HUVEC culture expressing a HUVEC-expressing fluorescent protein, such a GFP. The claims also recite continuously

culturing the HUVEC cultures for sufficient time for Rac to transfer into the nucleus. The cited art does not disclose or suggest this.

As to Krall, this reference does not disclose the use of a HUVEC culture expressing a HUVEC-expressing fluorescent protein and administration of a test substance. Rather, Krall discloses that an Exos gene was transfected together with GFP-Rac1 fusion protein into CHO cells, was co-expressed and immediately afterwards the observation was performed. From this process, the transfer of Rac into the nucleus cannot be observed, if HUVEC cells are used in place of CHO cells. This is because there is no disclosure of continuously culturing the cells in the presence of a test substance for sufficient time to transfer into the nucleus after introduction of Exos gene.

Furthermore, in Krall, the observation of Rac1 cellular location is merely the observation that when Exos is not expressed, GFP-Rac1 is mostly localized in membranes. Rather in Krall, it is disclosed that when Exos is expressed, GFP-Rac1 is activated and that this activated Rac1 is re-localized from the cell membrane to the cytoplasm. Since the claims recite observation of Rac1 transfer into the nucleus in order to prevent Rac1 from being activated (*i.e.*, for prevention of activated Rac1), Keller clearly differs in this respect from the claimed embodiments.

Furthermore, in Krall, the GST-Rac1 fusion protein in the HUVEC culture has been activated by adding S1P and the behavior of this activated Rac1 is observed. Therefore, like Keller, Essler discloses observing the actions of activated Rac1, rather than the observation of re-localization of Rac1. Essler does not disclose or suggest screening for a substance capable of removing Rac1 from the cell membrane in order to prevent Rac1 from being activated.

As shown at page 611, left column, third line from the bottom, to right column, line 7, the observation of activated Rac1 intercellular localization is performed a short time after adding S1P, such as 1-2 or 5 minutes after. Accordingly, Essler discloses observation of Rac1 in an activated state prior to the next signal transduction. Therefore, Essler also does not disclose or suggest the embodiments as claimed.

Additionally, Applicants note that Essler further differs from the claimed embodiments, which recite "continuously culturing" said first HUVEC culture and said second HUVEC culture for a sufficient time for the Rac fusion protein to transfer into the nucleus. Therefore, for at least the above reasons, Applicants respectfully submit that the combination of cited art does not disclose or suggest the embodiments as claimed. Favorable reconsideration is respectfully requested.

In view of the aforementioned amendments and accompanying remarks, Applicants submit that the claims, as herein amended, are in condition for allowance. Applicants request such action at an early date.

If the Examiner believes that this application is not now in condition for allowance, the Examiner is requested to contact Applicants' undersigned attorney to arrange for an interview to expedite the disposition of this case.

If this paper is not timely filed, Applicants respectfully petition for an appropriate extension of time. The fees for such an extension or any other fees that may be due with respect to this paper may be charged to Deposit Account No. 50-2866.

Respectfully submitted,

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Enclosures: Excerpt of USPTO Written Description Guidelines